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Original Article

Achromobacter species in cystic fibrosis: Cross-infection caused by indirect patient-to-patient contact☆

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Abstract

Background and methods: Achromobacter species leads to chronic infection in an increasing number of CF patients. We report 2 cases of *Achromobacter ruhlandii* cross-infection between patients after well-described indirect contact.
Results: Both cases were young, stable, CF patients without chronic infections and with normal FEV_1, but experienced clinical deterioration after visits to the home of a CF patient with *A. ruhlandii* infection and after sharing facilities with an *A. ruhlandii* infected CF patient on a skiing vacation, respectively. Both cases became positive for *A. ruhlandii* in airway secretions and were colonized with *A. ruhlandii* in their sinuses.

Aggressive, long-term antibiotic treatment led to clinical stability. One of the cases developed chronic *A. ruhlandii* infection.
Conclusion: A. species can cause cross-infection even after a short period of indirect contact between infected and non-infected CF patients. Patients should be followed closely for several months before the possibility of cross-infection is ruled out.

Keywords: Cross-infection; *Achromobacter xylosoxidans*; Pulmonary infection

1. Introduction

In cystic fibrosis (CF) patients, chronic, pulmonary infection caused by *Achromobacter species* is an increasing problem. Antibiotic resistance is common in *A. species* infection and develops early, making antibiotic treatment difficult. Although the prevalence of chronic *A. species* infection is low in most CF centers, chronic *A. species* infection may lead to rapid clinical deterioration in affected patients [2,3].

In the case of other Gram-negative infections in CF patients, cross-infection has been shown to take place with *Pseudomonas aeruginosa* [4–8] and *Burkholderia cepacia* complex (BCC) [9–13] and recommendations are to avoid close contact between chronically infected patients and patients without chronic, Gram-negative infections [14–16].

Transmission of *A. species* between CF patients has previously been reported [17]. Studies have shown transmission between closely related CF patients, i.e. siblings [18], or patients known to have had close, prolonged contact [3,19–23].

We present 2 cases of cross-infection between CF patients with no direct, but well-described indirect contact.

2. Methods

2.1. Study design

Clinical data used in this study were collected prospectively and stored in the Copenhagen CF Centre database from time of diagnosis and onwards.

☆ Data have been presented at the 35th European Cystic Fibrosis Conference in Dublin, June 2012, as abstract number 353 [1].

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After discovering the incidences of cross-infection described in this work, data were gathered retrospectively from the database.

2.2. Patients

All CF patients are seen on a regular, monthly basis. At all visits, the clinical status of the patients is assessed by weight, height and lung function parameters, forced vital capacity (FVC) and forced expiratory volume in 1s (FEV_1) (Masterscreen Pneumo, Jaeger) are obtained according to the American Thoracic Society guidelines, using reference values from Wang [24] and Hankinson et al. [25]. Lower respiratory tract secretions for microbiological investigation are obtained by coughing or by endo-laryngeal suctioning. Specific, precipitating antibodies are obtained at least once every year, in patients with a chronic lung infection every third month. Chronic infection is defined as more than 50% of positive sputum samples and/or elevated levels of specific, precipitating antibodies [26]. Intermittent colonization is defined as isolation of a CF related Gram-negative rod in less than 50% of the investigated sputum samples in the present year (*P. aeruginosa*, *A. species*, *Pandoraeae apista* or a BCC species) in a patient with no increase in specific antibodies. The diagnosis of CF is based on abnormal sweat electrolytes, characteristic clinical features and CFTR genotype.

2.3. Segregation policy

In the clinic, patients with CF-related chronic Gram-negative, pulmonary infections are isolated from patients without chronic infections, and patients are encouraged to avoid social interaction with other CF patients with chronic Gram-negative, pulmonary infections.

All horizontal surfaces are cleaned after an infected patient has been in a room, and the hygienic procedures are in accordance with the recommendations of the department of infection control in the hospital.

2.4. Pulsed field gel electrophoresis (PFGE)

Strains isolated from individual patients are stored on a yearly basis, and PFGE [27,28], using Spe I as restriction enzyme, is performed regularly to discover possible cases of cross-infection.

2.5. Multilocus sequence analysis

Isolates from the 2 cases and from the 2 chronically infected CF patients were investigated using multilocus sequence analysis [17] and compared to a reference strain of *Achromobacter ruhlandii*, the Danish epidemic strain that has infected multiple patients at Danish CF centers in Aarhus and Copenhagen [3,17,23], as previously described.

3. Results

3.1. Case 1: A girl born in April 1999

Diagnosis was made shortly after birth due to meconium ileus. Her CFTR genotype is ΔF508 homozygous and she has been followed with monthly controls in the outpatient clinic since diagnosis. Daily treatment included pancreatic enzyme replacement since birth, lung physiotherapy and from the age of 21 months, also daily Pulmozyme® inhalation.

Monthly endolaryngeal suctioning revealed intermittent colonization with *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* and occasionally *Staphylococcus aureus*. All infections were treated with 2 week courses of oral antibiotics.

Case 1 had had 2 isolates of *P. aeruginosa*. First ever isolate of *P. aeruginosa* was cultured in April 2006 and treated with 4 weeks of inhaled colomycin and oral ciprofloxacin. Second isolate of *P. aeruginosa* was cultured in March 2007 and was treated with 3 months of inhaled colomycin and oral ciprofloxacin. This time, the patient had an asthmatic reaction to colomycin and received bronchodilator-treatment.

Measurement of lung function was performed since the age of 5.5 years and was stable with FEV_1 above 100 % predicted (Fig. 1).

The girl lives in a small village on an isolated island. In 2006, another CF patient moved to the village with her family, including 2 twin sisters (non-CF) of the same age as Case 1. The 3 girls became friends and Case 1 came in the home of the 2 twin sisters and their 9 years older sister with CF and chronic *A. ruhlandii* infection, the Danish epidemic strain. The parents took great care never letting the 2 CF patients stay in a room simultaneously.

After completion of 3 months of anti-*P. aeruginosa* treatment in June 2007, Case 1 experienced weight-loss and a dramatic decline in lung function and showed no effect of bronchodilator-treatment.

Within a few weeks, sputum samples became positive for multi-resistant *A. species*, only sensitive to sulfometoxazole. PFGE showed that the *A. ruhlandii* strains from Case 1 and from the older CF patient were identical, indicating patient-to-patient transmission, and multilucos sequence analysis confirmed that Case 1 harbored the Danish epidemic strain (Fig. 3).

In April 2008, functional endoscopic sinus surgery (FESS) was performed and *A. ruhlandii* was cultured from the sinuses.

Inhaled ceftazidime and oral sulfometoxazole and trimetoprim were started immediately after culture of first isolate of *A. ruhlandii*. Inhaled ceftazidime has been given continuously since then and supplemented with courses of oral antibiotics and/or iv. antibiotics. In August 2008, inhaled antibiotics were changed to colomycin, but again the patient had an asthmatic reaction with a dramatic decline in lung function. Lung function immediately stabilized when treatment was switched back to ceftazidime.

The patient is clinically stabilized, but sputum cultures remain positive for *A. ruhlandii* and specific, precipitating anti-*A. species* antibodies have increased to a steady level around 7–10, and the patient is considered chronically infected.

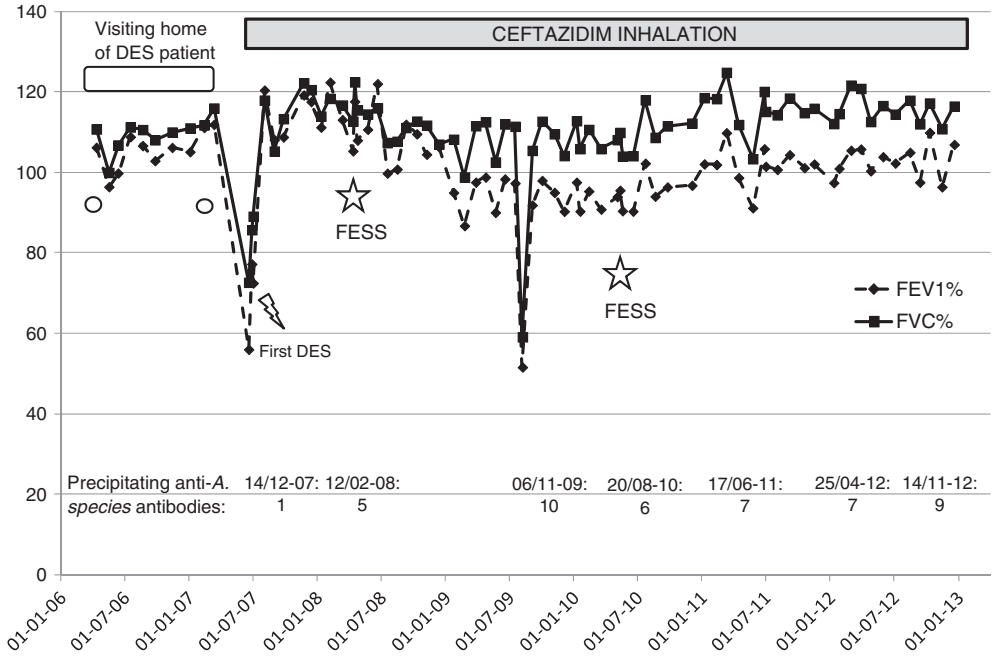


Fig. 1. Case 1, lung function, microbiology and treatment from 2006 until January 2013. FEV_1 and FVC are shown as % of predicted. Top bars show period of possible transmission (open) and period of antibiotic treatment (gray, shown as ceftazidime inhalation). Lightning shows first isolation of *A. ruhlandii* (Danish Epidemic Strain). Five-pointed star shows FESS. Open circles show intermittent colonization with *P. aeruginosa*. At the bottom, level of specific, precipitating anti-*A. species* antibodies is shown at different time-points.

3.2. Case 2: A boy born in December 1997

Diagnosis was made shortly after birth due to meconium ileus. Sweat test was positive with sodium-concentration above 80 mmol/l. CF genotype was difficult to identify, but the boy, who is of Pakistani origin, is heterozygous with mutations del ex 11,12 and del ex 12,13,14A, respectively. These mutations have not been classified, but the phenotype of this patient is classical CF. During the first 2 years of life, the boy had compliance and nutritional problems. Following replacement in an institution his clinical condition improved. Daily treatment included pancreatic enzyme replacement since birth, lung physiotherapy and from the age of 4.5 years, also daily Pulmozyme® inhalation.

Monthly cultures obtained by endo-laryngeal suctioning revealed intermittent colonization with *H. influenzae*, *S. aureus*, and *M. catarrhalis*. All infections were treated with 2 week courses of oral antibiotics.

Case 2 had only had *P. aeruginosa* once in May 2007, treated with 3 months of inhaled colomycin and oral ciprofloxacin.

Measurement of lung function was performed since the age of 6.5 years and was stable with FEV_1 around 80% of predicted (Fig. 2).

In January 2011, the boy went on a one week skiing holiday with the institution in which he lives. Another CF patient, 22 years old, chronically infected with the Danish epidemic strain of *A. ruhlandii*, also attended the ski-camp. The 2 CF patients did not have close contact and slept in separate rooms, but shared dining and living facilities.

Over the next 6 months, Case 2 experienced fatigue and an ongoing decline in lung function, but no increase in cough or sputum production. Sputum samples grew *H. influenzae* once, but were otherwise negative. Chest X-ray revealed increased air-trapping. Blood samples showed numbers of specific, precipitating anti-*A. species* antibodies increasing from 0 to 6.

Broncho-alveolar lavage (BAL) was performed in July 2011 in order to hopefully explain the symptoms. The BAL-fluid showed multi-resistant *A. species*, only partially sensitive to piperacillin with tazobactam, ciprofloxacin, imipenem, tigecycline and chloramphenicol.

PFGE showed that strains from Case 2 and the adult CF patient were identical, indicating that transmission and multilucos sequence analysis confirmed that Case 2 harbored the Danish epidemic strain (Fig. 3).

In October 2011, FESS was performed and *A. ruhlandii* was cultured in secretions from the sinuses.

Immediately after culture of *A. ruhlandii* in BAL-fluid, antibiotic treatment was initiated with a course of iv. piperacillin with tazobactam and oral ciprofloxacin. After finishing this course, the patient has been inhaling ceftazidime continuously and is having 2 weeks iv.-courses every 3 months.

Lung function has stabilized on antibiotic treatment, and the patient has not yet had growth of *A. ruhlandii* in spontaneously expectorated sputum, but levels of specific, precipitating anti-*A. ruhlandii* antibodies remained increased in 2011, but have been decreasing in 2012 to 1 during constant ceftazidime-inhalation. This patient does not meet the criteria for chronic infection at the present time.

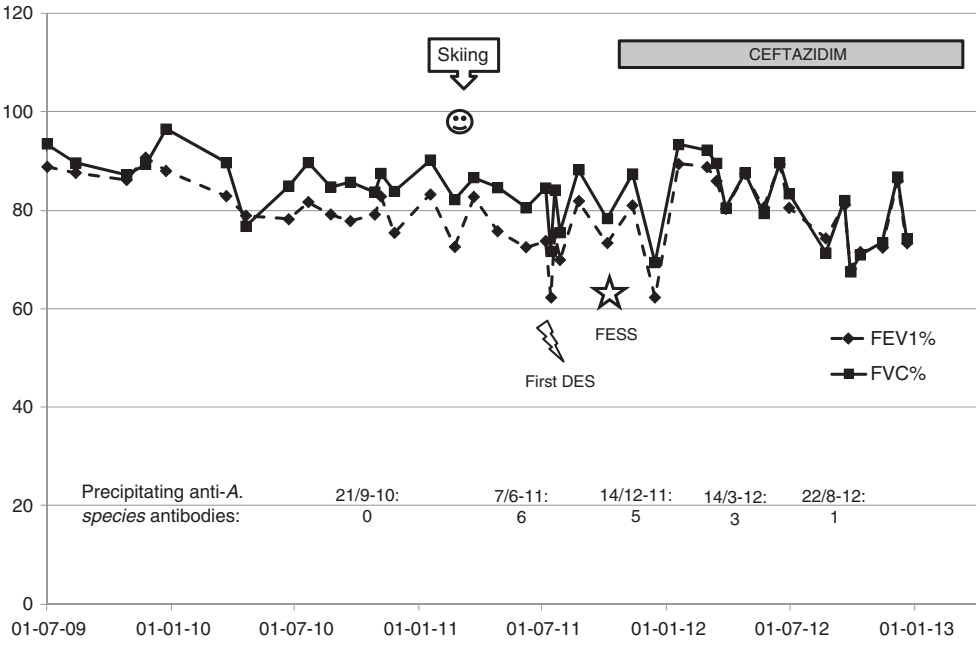


Fig. 2. Case 2, lung function, microbiology and treatment from 2009 until January 2013. FEV_1 and FVC are shown as % of predicted. Top bars show period of possible transmission and period of antibiotic treatment (shown as ceftazidime inhalation). Lightning shows first isolation of *A. ruhlandii* (Danish Epidemic Strain). Five-pointed star shows sinus-surgery. At the bottom, level of specific, precipitating anti-*A. species* antibodies is shown at different time-points.

4. Discussion

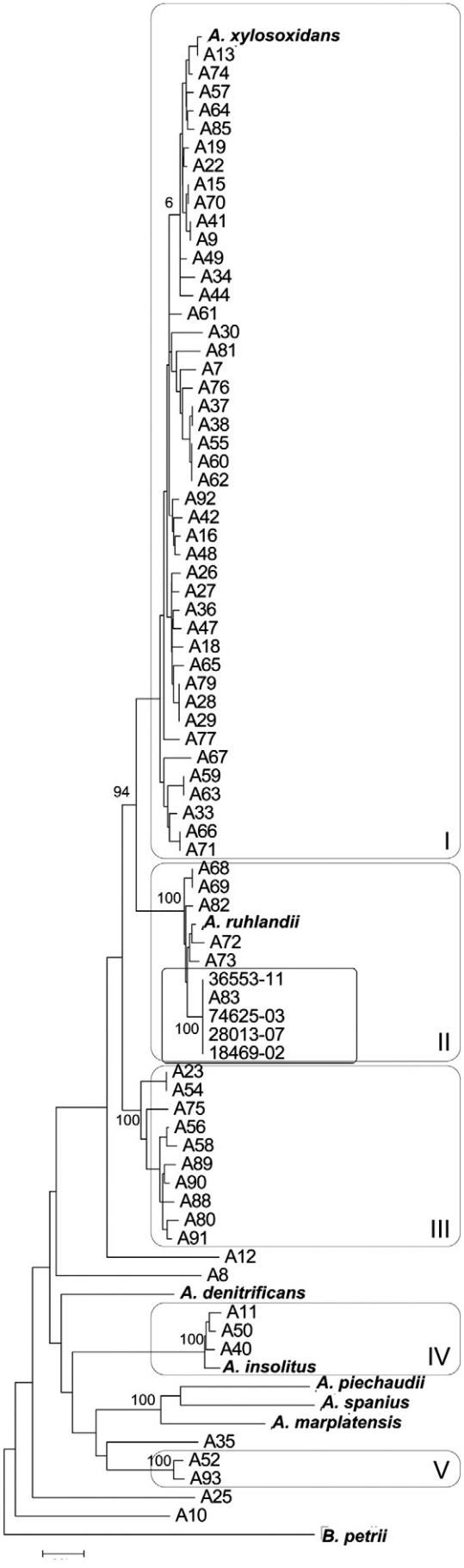
The incidence of chronic, pulmonary *A. species* infection in CF patients has increased over the past few years. Several cases of cross-infections have been described, but until now, cross-infection has only been known to happen after close, prolonged contact between CF patients with chronic *A. species* infection and patients without infection.

In the present work, two clinically stable CF patients with normal lung function, without chronic, Gram-negative, pulmonary infections acquired *A. ruhlandii* after indirect contact to other CF patients chronically infected with the Danish epidemic strain of *A. ruhlandii*. Interestingly, both patients experienced clinical deterioration for a few months before *A. ruhlandii* was isolated, suggesting that clinical symptoms due to *A. ruhlandii* are possible even in the very early stages of a chronic infection. No other cause of the clinical deterioration was found in either patient, and in Case 2, *A. ruhlandii* was not found until a BAL was performed. To our knowledge, no other studies have presented clinical symptoms so shortly after acquiring the infection.

The finding of multi-resistant strains was very suspicious for transmission. Although *A. species* develops multi-resistance against antibiotics very fast, initial isolates are often antibiotic sensitive, leaving a few different treatment options [29,30]. However, no direct contact between the cases and the chronically infected CF patients had occurred to our knowledge. These patients were not of a similar age, and cross infection was not expected initially, since the segregation policy of the Copenhagen CF center does not allow patients with chronic *A. species* infection to be in contact with non-infected patients. Only after the PFGE results, showing identical strains in the cases and respective chronically infected CF patients, were the circumstances regarding possible transmission explored. The mechanism of transmission in our cases is probably due to the presence of *A. ruhlandii* on horizontal surfaces, door handles or in aerosols, since the patients did not have direct contact with each other. It has been shown that CF pathogens can be cultured from the surroundings of CF patients, originating from cough-generated aerosols [31]. Peltroche-Llacsahuanga et al. describe 2 CF brothers in whom the younger brother only developed chronic *A. species* infection 3 years after his older brother [18]. The brothers lived together, and transmission was expected to occur earlier.

A group of Danish teenagers have shared the same *A. species* strain for several years (the Danish epidemic strain) [3,23]. This strain was recently identified as *A. ruhlandii* [17]. The affected patients are known to have had close, social contact over several years outside the hospital, and cross-infection occurred before the segregation policy for *A. species* was introduced in the Danish CF centers. Why this specific strain became epidemic has not yet been clarified. Other strains of *A. species* have been shown to lead to cross-infection [17], but it is noteworthy that it was the Danish epidemic strain leading to cross-infection after indirect contact for a shorter time period.

In a study by van Daele et al., transmission of not only *A. species* [20], but also *P. aeruginosa* [32] took place at a Belgian rehabilitation center. In the center, CF patients lived in separate rooms, but shared dining and living facilities. The patients stayed at the center for a median of 63 days [32], and not everyone was colonized with the transmissible strains after the stay. These results add to the evidence of transmission needing time to occur.



In a study by Pereira et al., 39 out of 179 CF patients at some point harbored *A. species* [22]. Five patients were chronically infected, among these 2 sets of siblings sharing identical strains. A total of 22 of the 39 included patients at some point shared the same *A. species* clone. Authors suggest that transmission possibly occurred during visits to the hospital. This is the first study describing *A. species* cross infection in a larger group of CF patients.

De Baets et al. [2] found that patients developing chronic *A. species* infection had worse lung function and radiological evidence of more pronounced lung damage on chest X-rays and high resolution CT scans compared to CF patients not developing chronic *A. species* infection and suggest that chronic *A. species* infection mainly develop in CF patients with increased pulmonary damage [33]. In several studies, chronic *P. aeruginosa* infection was established prior to the development of chronic *A. species* infection [19,33–38], and may be the cause of significant pulmonary damage increasing the risk of development of other chronic, pulmonary infections. In 2 studies published by our own group, the majority of CF patients with chronic *A. species* infection did not harbor any co-infections, however [3,39].

In our study, *A. ruhlandii* was not found in the Case 2 until several months after contact to infected patient. This shows the importance of frequent, clinical and microbiological follow-up in CF patients in order to discover new infections, but also before the possibility of cross infection can be ruled out.

In both cases, intensive endoscopic sinus surgery revealed *A. ruhlandii* in sinus secretions and it is possible that sinus colonization with *A. species* preceded the development of pulmonary infection.

Focus on sinuses as a bacterial reservoir (united airways) has increased in CF patients [40], and newer studies support the theory of chronic, pulmonary *P. aeruginosa* infections beginning as rhino-sinusitis, dripping bacteria to the lower airways, thereby leading to pulmonary infection [41–43]. In one of our cases, *A. ruhlandii* was found exclusively in sinus secretions and in BAL-fluid but not in sputum, although the patient experienced clinical deterioration and the level of precipitating antibodies increased. CF patients with unexplained lung function decline and clinical deterioration should undergo thorough investigation – especially if sputum samples are negative – in order to diagnose possible lower airway or sinus infection. These investigations should include sinus examination, and infection can only be ruled out if sinus samples as well as BAL-fluid cultures are negative.

In a new study by Wang et al. (manuscript submitted), use of inhaled antibiotics was found to be valuable in clearing *A. species* infection in CF patients with first ever isolate. The use of continuous inhaled antibiotics in the 2 cases described here is possibly an important reason for the clinical stability of the cases, even after fulfilling the criteria for development of chronic infection.

Fig. 3. Multilocus sequence analysis showing the 4 isolates from the cases and the chronically infected patients. These 4 isolates cluster with the Danish epidemic strain of *A. ruhlandii*, strain A83.

5. Conclusion

A. species is capable of causing cross infection even if direct contact between patients is avoided. If known indirect contact has happened, the patient should be followed closely for several months before the possibility of cross-infection is ruled out. Symptoms of pulmonary exacerbation do not necessarily present and the occurrence of *A. species* in sputum cultures can be delayed for weeks after cross-infection.

Like *P. aeruginosa*, *A. species* can colonize the sinuses and when evaluating a CF patient experiencing clinical deterioration, examination of the sinuses should be part of the assessment, since chronic rhinosinusitis might lead to chronic, lower airway infection in these patients.

A. species is capable of causing cross-infection even after short, indirect contact between infected and non-infected CF patients.

References

[1] Hansen CR, Pressler T, Johansen HK, Skov M. *Achromobacter xylosoxidans* in cystic fibrosis: indirect patient-to-patient contact can lead to cross-infection.J Cyst Fibros 2012;11(Supplement 1):S147 [Ref Type: Abstract].

[2] Romano L, Bellodi S, Lugani F, Manno G. To the editor: alcaligenes as a pathogen in airways chronic infection in cystic fibrosis. Pediatr Pulmonol May 2003;35(5):412–3.

[3] Rønne Hansen C, Pressler T, Høiby N, Gormsen M. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients: a retrospective case control study. J Cyst Fibros Dec 2006;5(4):245–51.

[4] Denton M, Kerr K, Mooney L, Keer V, Rajgopal A, Brownlee K, et al. Transmission of colistin-resistant *Pseudomonas aeruginosa* between patients attending a pediatric cystic fibrosis center. Pediatr Pulmonol Oct 2002;34(4): 257–61.

[5] Frederiksen B, Koch C, Høiby N. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974–1995). Pediatr Pulmonol Sep 1999;28(3):159–66.

[6] Høiby N, Pedersen SS. Estimated risk of cross-infection with *Pseudomonas aeruginosa* in Danish cystic fibrosis patients. Acta Paediatr Scand May 1989;78(3):395–404.

[7] Ojeniyi B, Frederiksen B, Høiby N. *Pseudomonas aeruginosa* cross-infection among patients with cystic fibrosis during a winter camp. Pediatr Pulmonol Mar 2000;29(3):177–81.

[8] Pedersen SS, Koch C, Høiby N, Rosendal K. An epidemic spread of multiresistant *Pseudomonas aeruginosa* in a cystic fibrosis centre. J Antimicrob Chemother Apr 1986;17(4):505–16.

[9] LiPuma JJ, Marks-Austin KA, Holsclaw Jr DS, Winnie GB, Gilligan PH, Stull TL. Inapparent transmission of *Pseudomonas* (*Burkholderia*) *cepacia* among patients with cystic fibrosis. Pediatr Infect Dis J Aug 1994;13(8):716–9.

[10] Mahenthiralingam E, Baldwin A, Vandamme P. *Burkholderia cepacia* complex infection in patients with cystic fibrosis. J Med Microbiol Jul 2002;51(7):533–8.

[11] Muhdi K, Edenborough FP, Gumery L, O'Hickey S, Smith EG, Smith DL, et al. Outcome for patients colonised with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. Thorax Apr 1996;51(4):374–7.

[12] Whiteford ML, Wilkinson JD, McColl JH, Conlon FM, Michie JR, Evans TJ, et al. Outcome of *Burkholderia* (*Pseudomonas*) cepacia colonisation in children with cystic fibrosis following a hospital outbreak. Thorax Nov 1995;50(11):1194–8.

[13] Burns JL. *Burkholderia cepacia*—a transmissible cystic fibrosis pathogen. J Pediatr Nov 2001;139(5):618–9.

[14] Döring G, Høiby N. Early intervention and prevention of lung disease in cystic fibrosis: a European consensus. J Cyst Fibros Jun 2004;3(2):67–91.

[15] France MW, Dodd ME, Govan JR, Doherty CJ, Webb AK, Jones AM. The changing epidemiology of Burkholderia species infection at an adult cystic fibrosis centre. J Cyst Fibros Sep 2008;7(5):368–72.

[16] Saiman L, Siegel J. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. Infect Control Hosp Epidemiol May 2003;24(5 Suppl.):S6–S52.

[17] Ridderberg W, Wang M, Norskov-Lauritsen N. Multilocus sequence analysis of isolates of *Achromobacter* from patients with cystic fibrosis reveals infecting species other than *Achromobacter xylosoxidans*. J Clin Microbiol Aug 2012;50(8):2688–94.

[18] Peltroche-Llacsahuanga H, Haase G, Kentrup H. Persistent airway colonization with *Alcaligenes xylosoxidans* in two brothers with cystic fibrosis. Eur J Clin Microbiol Infect Dis Feb 1998;17(2):132–4.

[19] Kanellopoulou M, Pournaras S, Iglezos H, Skarmoutsou N, Papagrangas E, Maniatis AN. Persistent colonization of nine cystic fibrosis patients with an *Achromobacter* (*Alcaligenes*) *xylosoxidans* clone. Eur J Clin Microbiol Infect Dis Apr 2004;23(4):336–9.

[20] van Daele S, Verhelst R, Claeys G, Verschraegen G, Franckx H, van Simaey L, et al. Shared genotypes of *Achromobacter xylosoxidans* strains isolated from patients at a cystic fibrosis rehabilitation center. J Clin Microbiol Jun 2005;43(6):2998–3002.

[21] Raso T, Bianco O, Grosso B, Zucca M, Savoia D. *Achromobacter xylosoxidans* respiratory tract infections in cystic fibrosis patients. APMIS Sep 2008;116(9):837–41.

[22] Pereira RH, Carvalho-Assef AP, Albano RM, Folescu TW, Jones MC, Leao RS, et al. *Achromobacter xylosoxidans*: characterization of strains in Brazilian cystic fibrosis patients. J Clin Microbiol Oct 2011;49(10): 3649–51.

[23] Ridderberg W, Bendstrup KE, Olesen HV, Jensen-Fangel S, et al. Marked increase in incidence of *Achromobacter xylosoxidans* infections caused by sporadic acquisition from the environment. J Cyst Fibros Dec 2011;10(6): 466–9.

[24] Wang X, Dockery DW, Wypij D, Fay ME, Ferris Jr BG. Pulmonary function between 6 and 18 years of age. Pediatr Pulmonol Feb 1993;15(2):75–88.

[25] Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med Jan 1999;159(1):179–87.

[26] Høiby N, Flensborg EW, Beck B, Friis B, Jacobsen SV, Jacobsen L. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. Scand J Respir Dis Apr 1977;58(2):65–79.

[27] Ojeniyi B, Høiby N, Rosdahl VT. Genome fingerprinting as a typing method used on polyagglutinable *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. APMIS Jun 1991;99(6):492–8.

[28] Johansen HK, Moskowitz SM, Ciofu O, Pressler T, Høiby N. Spread of colistin resistant non-mucoid *Pseudomonas aeruginosa* among chronically infected Danish cystic fibrosis patients. J Cyst Fibros Sep 2008;7(5):391–7.

[29] Magni A, Giordano A, Mancini C, Pecoraro C, Varesi P, Quattrucci S, et al. Emerging cystic fibrosis pathogens: incidence and antimicrobial resistance. New Microbiol 2007 Jan;30(1):59–62.

[30] Saiman L, Chen Y, Tabibi S, San Gabriel P, Zhou J, Liu Z, et al. Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. J Clin Microbiol Nov 2001;39(11):3942–5.

[31] Wainwright CE, France MW, O'Rourke P, Anuj S, Kidd TJ, Nissen MD, et al. Cough-generated aerosols of *Pseudomonas aeruginosa* and other bacteria from cystic fibrosis patients. Thorax Jul 1 2009.

[32] Van Daele SG, Franckx H, Verhelst R, Schelstraete P, Haerynck F, Van Simaey L, et al. Epidemiology of *Pseudomonas aeruginosa* in a cystic fibrosis rehabilitation centre. Eur Respir J Mar 2005;25(3): 474–81.

[33] de Baets F, Schelstraete P, van Daele S, Haerynck F, Vaneechoutte M. *Achromobacter xylosoxidans* in cystic fibrosis: prevalence and clinical relevance. J Cyst Fibros Jan 2007;6(1):75–8.

[34] Lambiase A, Catania MR, Del PM, Rossano F, Terlizzi V, Sepe A, et al. *Achromobacter xylosoxidans* respiratory tract infection in cystic fibrosis patients. Eur J Clin Microbiol Infect Dis Aug 2011;30(8):973–80.
[35] Tan K, Conway SP, Brownlee KG, Etherington C, Peckham DG. *Alcaligenes* infection in cystic fibrosis. Pediatr Pulmonol Aug 2002;34(2):101–4.
[36] Dunne Jr WM, Maisch S. Epidemiological investigation of infections due to *Alcaligenes* species in children and patients with cystic fibrosis: use of repetitive-element-sequence polymerase chain reaction. Clin Infect Dis Apr 1995;20(4):836–41.
[37] Moissenet D, Baculard A, Valcin M, Marchand V, Tournier G, Garbarg-Chenon A, et al. Colonization by *Alcaligenes xylosoxidans* in children with cystic fibrosis: a retrospective clinical study conducted by means of molecular epidemiological investigation. Clin Infect Dis Feb 1997;24(2):274–5.
[38] Spicuzza L, Sciuto C, Vitaliti G, Di Dio G, Leonardi S, La Rosa M. Emerging pathogens in cystic fibrosis: ten years of follow-up in a cohort of patients. Eur J Clin Microbiol Infect Dis Feb 2009;28(2):191–5.
[39] Hansen CR, Pressler T, Nielsen KG, Jensen PO, et al. Inflammation in *Achromobacter xylosoxidans* infected cystic fibrosis patients. J Cyst Fibros Jan 2010;9(1):51–8.
[40] Aanaes K, von Buchwald C, Hjuler T, Skov M, Alanin M, Johansen HK. Extensive sinus surgery and follow-up can eradicate pathogenic bacterial sinusitis in patients with cystic fibrosis.J Cyst Fibros 2012;11(Supplement 1):S53 [Ref Type: Abstract].
[41] Roby BB, McNamara J, Finkelstein M, Sidman J. Sinus surgery in cystic fibrosis patients: comparison of sinus and lower airway cultures. Int J Pediatr Otorhinolaryngol Sep 2008;72(9):1365–9.
[42] Johansen HK, Aanaes K, Pressler T, Nielsen KG, Fisker J, Skov M, et al. Colonisation and infection of the paranasal sinuses in cystic fibrosis patients is accompanied by a reduced PMN response. J Cyst Fibros May 15 2012.
[43] Hansen SK, Rau MH, Johansen HK, Ciofu O, et al. Evolution and diversification of *Pseudomonas aeruginosa* in the paranasal sinuses of cystic fibrosis children have implications for chronic lung infection. ISME J Jan 2012;6(1):31–45.